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Release of carbon nanoparticles of different size and shape from nanocomposite poly(lactic) acid film into food simulants

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ABSTRACT

Poly(lactic) acid (PLA) film with 2 wt% mixed carbon nanofillers of graphene nanoplates (GNPs) and multiwall carbon nanotubes (MWCNTs) in a weight ratio of 1:1 with impurities of fullerene and carbon black (CB) was produced by layer-to-layer deposition and hot pressing. The release of carbon nanoparticles from the film was studied at varying time–temperature conditions and simulants. Migrants in simulant solvents were examined with laser diffraction analysis and transmission electron microscopy (TEM). Film integrity and the presence of migrants on the film surfaces were visualised by scanning electron microscopy (SEM). The partial dissolution of PLA polymer in the solvents was confirmed by swelling tests and differential scanning calorimetry (DSC). Nanoparticle migrants were not detected in the simulants (at the LOD 0.020 μm of the laser diffraction analysis) after migration testing at 40°C for 10 days. However, high-temperature migration testing at 90°C for 4 h provoked a release of GNPs from the film into ethanol, acetic acid and oil-based food simulants. Short carbon nanotubes were observed rarely to release in the most aggressive acetic acid solvent. Obviously, the enhanced molecular mobility at temperatures above the glass transition and partial dissolution of PLA polymer by the food simulant facilitate the diffusion processes. Moreover, shape, size and concentration of nanoparticles play a significant role. Flexible naked GNPs (lateral size 100–1000 nm) easily migrate when the polymer molecules exhibit enhanced mobility, while fibrous MWCNTs (> 1 μm length) formed entangled networks on the film surfaces as the PLA polymer is partly dissolved, preventing their release into food simulants. The impurities of fullerenes and CB (5–30 nm) were of minor concentration in the polymer, therefore their migration is low or undetectable. The total amount of released migrants is below overall migration limits.

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Introduction

Applications of nanomaterials in active and intelligent food packaging are rapidly becoming a commercial reality and already make up the largest share of the current and short-term-predicted food market (Chaudhry et al. 2008; Ayhan 2013; Cui et al. 2016). Incorporation of graphene and carbon nanotubes (1–2 wt %) in polymers is a promising approach for multifunctional food packaging applications. Compared with clay nanofiller, this gives rise to several benefits, such as improved mechanical performance (Ivanov & Kotsilkova 2015) and antimicrobial properties (Dizaj et al. 2015), and also one can trace and monitor the condition of food during transport and storage (de Azeredo et al. 2011). Graphene and its derivatives are identified as

powerful candidates for gas-barrier materials because perfect graphene does not allow the diffusion of small gases through its plane (Du & Cheng 2012; Cui et al. 2016). Recently, poly(lactic) acid (PLA) has received attention as a sustainable, biocompatible, biodegradable material with good mechanical and optical properties (Jamshidian et al. 2010). However, the large-scale use of PLA as a packaging material is hindered by its poor gas-barrier properties compared with commodity polymers that may be improved by nanocomposites technology (Wu et al. 2014). Incorporation of graphene and carbon nanotubes into PLA is expected not only to enhance gas-barrier properties but also to increase mechanical strength and improve thermal properties when properly dispersed in a polymer matrix

(Huang et al. 2014). The use of two-dimensional graphene nanoplates (GNPs) as a surface coating instead of bulk additives overcomes common issues related to the dispersion of nanofiller in a polymer matrix, and gives a clear advantage in preserving the mechanical properties of the bulk polymer (Pierleoni et al. 2016). Such graphene-based coatings placed on the surface of several industrially relevant commodity polymers significantly improve the gas-barrier properties of polymeric films for large-scale applications. However, to our knowledge it is not obvious in the reviewed literature whether graphene and carbon nanotubes can migrate into food from polymeric films and what is the potential hazard after such migration.

A contributing factor to the rapid commercial development in polymer nanocomposite food packaging materials is the expectation that, due to the fixed or embedded nature of nanoparticles in polymer, they will not pose any significant risk to the consumer (Chaudhry et al. 2008). Recent research on nanoparticle migration from packaging film into foodstuffs shows contradicting results. From both experimental findings and theoretical modelling, Bott et al. (2014) concluded that carbon black (CB) does not migrate into food once it is incorporated into low-density polyethylene and polystyrene-based films, when test conditions of 10 days at 60°C were applied. Schmidt et al. (2009, 2011) found that asymmetrical nanoclay layers of lateral size 50–800 nm embedded in PLA nanocomposites indeed migrate from the nanocomposite in 95% ethanol after 10 days at 40°C, and this was attributed to the weak filler to polymer interfacial interactions. Lin et al. (2014) found that the migration of Ti from nano-TiO₂-polyethylene packaging films into food simulants might occur via dissolution from the surface and the cut edges of the film under different temperature and migration time conditions. Detailed investigations on the effect of high-pressure thermal treatments (e.g., pasteurisation and sterilisation) on food/packaging interactions focusing on migration from the PLA/gluten/montmorillonite nanocomposite materials into food stuff found that the overall migration and protein migration were high, while the migration of montmorillonite was low or undetectable (Mauricio-Iglesias et al. 2010, 2010a). The reported results lead to the conclusion that a partial migration of nanoparticles of different size and

shape from packaging films into food or food simulant cannot be excluded. Therefore, the confirmation of the estimated migration by experimental testing is obligatory in order to demonstrate the applicability of a nanomaterial as a food-contact material.

Migration modelling predicted that spherical nanoparticles larger than 1 nm in diameter cannot migrate, following Fickian laws of diffusion from polymer matrices. Franz (2015) and Simon et al. (2008) stated, based on the diffusion modelling approach, that any detectable migration of engineering nanoparticles from packaging film to food will take place in the case of very small particles with a radius in the order of 1 nm (e.g., TiN and Ag), from polyolefin-based matrices (LDPE, HDPE, PP) that have a relatively low dynamic viscosity, and which do not interact with the nanoparticles. Duncan and Pillai (2015) considered two nanoparticle release paradigms: (1) via passive diffusion, desorption and dissolution into external liquid media; and (2) by matrix degradation. However, it is still not explored in depth if swelling of the polymer at the film surface and partial dissolution of some organic ingredients may cause the physical release of nanoparticles of different size, shape and entanglement, from plastic films towards foods/or food simulants.

If one considers PLA packaging films, ethanol and polar solvents are found to be aggressive to the PLA films due to polymer hydrolysis leading to dissolution of lactic acid-based organic substances that are hydrolysed in aqueous systems to lactic acid (Jamshidian et al. 2010). Mutsuga et al. (2008) reported that the rate of migrate of lactic acid products from PLA polymer into food simulants is augmented by high temperatures and long-term tests; thus, sorption of certain organic solvents could cause the dissolution of one or more components of the polymer matrix. Fortunati et al. (2012) found that the polymer degradation and migration level may be controlled below the overall migration limits (OMLs) by the incorporation of nanofillers, such as cellulose nanocrystals, in the PLA film.

To answer the needs for greater information on the release and potential risk associated with nanoparticles for food-packaging applications, the objective of this study was to assess whether the embedded carbon nanofillers of different size and shape can transfer in nanoform from polymeric film into food-simulating solutions under different

migration conditions. To process polymeric films, three-dimensional printing-fused deposition modelling (FDM) was used, a new technique with the potential to be utilised in the packaging industry for rapid prototyping of food containers (Lingle 2015). PLA-based polymer nanocomposite incorporating mixed-carbon nanoparticles (including graphene, carbon nanotubes, fullerenes and CB) was chosen in order to study how the distinct geometric shapes, aspect ratios and concentration of nanofiller affect the nanoparticle release from the film into the food simulant. The swelling and dissolution of the polymer film into acidic-, ethanol- and oil-based food simulants under various time-temperature migration conditions were studied by the swelling test, SEM and differential scanning calorimetry (DSC). The migrants from the polymeric films released into the food simulants were detected by a laser nanoparticles sizer and transmission electron microscopy (TEM). Safety concerns are discussed for consumers exposed to GNPs released into food and drinks over the long-term.

Materials and methods

Materials

Commercial poly(lactic) acid composite doped with graphene and carbon nanofillers (GRPHN-175) was supplied by Graphene 3D Lab (Calverton, NY, USA). The neat poly(lactic) acid polymer (PLA), Ingeo Biopolymer 404 3D, supplied by NatureWorks (Minnetonka, MN, USA) was used as a control material. The PLA nanocomposite was produced by melt extrusion to a filament of size 1.75 mm. Carbon nanofillers in the PLA composite

are graphene and multiwall carbon nanotubes (MWCNTs) in a weight ratio of 1:1, with traces of impurities of fullerenes and carbon black (CB). Graphene nanoplates (GNPs) with lateral size of 100–1000 nm and thickness of 5–6 nm, as well as MWCNTs with length around 1000 nm and outer diameter of 10–30 nm, are the main nanofiller of the PLA nanocomposite, as seen from the TEM analysis (Figure 1(a)). Traces of fullerenes (5–10 nm) and CB (approximately 30 nm) were identified as impurities by the TEM analysis. The surfaces of carbon nanofillers were not functionalised with chemical groups.

The 3D printing, fused-deposition modelling technique (Górski et al. 2013; Lingle 2015) was applied for processing of the films by layer-to-layer deposition of alternating a composite PLA layer and a neat PLA layer. The 3D printed films of 500 μm thickness were hot pressed to produce the final film of about 30 μm thickness. The total amount of the carbon nanofiller in the films was about 2 wt%. Figure 1(b) shows the SEM micrograph of the cross-section of the PLA-nanocomposite film visualising the homogeneous dispersion of carbon nanofillers in the matrix polymer. The test samples were further indicated as the nanocomposite PLA/GR/NC film and the neat PLA film used as a control.

Migration tests

The migration test involves two steps. First, the film was immersed into the food simulant(s) allowing the film to absorb the food simulant, as well as substances from the packaging material to migrate into the simulant(s) at definite time-temperature conditions. The second was to detect the carbon nanofiller migrants transferred into a food simulant in terms of

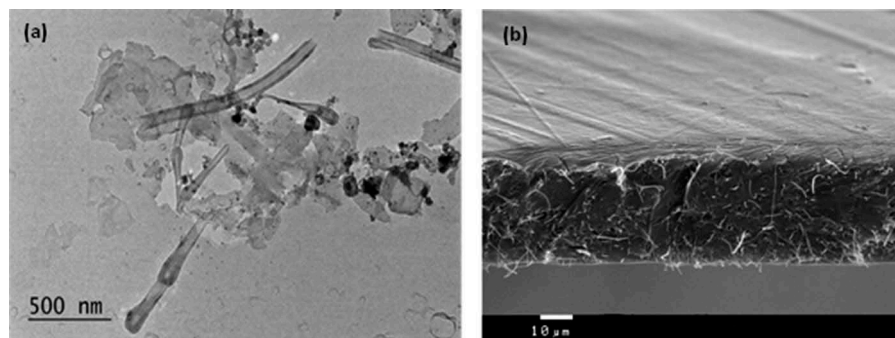


Figure 1. TEM micrograph of the PLA/GR/NC nanocomposite film doped with graphene and carbon nanotubes with traces of impurities like fullerenes and carbon black (a); and SEM micrograph of the cross section of the film (b).

specific migration, according to the European Standard EN 13130-1:2004 (EN 2004). In this study we were interested in detecting carbon nanofillers as specific migrants that could migrate from the composite film into the simulant solutions. Film samples were thin round plates with a diameter of about 3 cm and thickness of 30 μm . The total film surface-to-simulant volume ratio was about 14 cm^2 of film-contact area totally immersed in 30 ml food simulant. Four samples were tested for each of the simulants. Pretreatment of test specimens for the removal of dust was performed by washing in distilled water and drying (JRC 2009). The migrant transferred from the films into the food simulants was detected by laser diffraction analysis and TEM.

Four standard food simulants were used in this study: ethanol/water 10% (v/v) as a simulant for aqueous foods (simulant A); acetic acid/water 3% (v/v) for acidic foods (simulant B); and 50% (v/v) ethanol/water (simulant D1) for alcoholic drinks and also milk, as well as olive oil (simulant D2) for fatty foods, as prescribed in EU Regulation 10/2011 (EU 2011).

Migration tests were performed in a temperature-controlled chamber, under static and dynamic conditions, as follows: (1) 10 days at 40°C (standard static test); (2) 4 h at 90°C (high-temperature static test), and (3) 4 h at 90°C and subsequent storage for 10 days at 40°C, including ultrasonic treatment 5 min daily in an ultrasonic bath of power 250 W (combined high-temperature-long-term dynamic test). The standard static test was set accordingly with the prescription in EU Regulation 10/2011 (EU 2011). The high-temperature static and dynamic migration conditions were chosen based on literature sources (Mutsuga et al. 2008; Xu et al. 2010), the aim being to mimic the use of nanocomposite films in extreme conditions, such as high-temperature processing, including microwaving, and subsequent long-term storage and transportation. In our study we investigate if different time-temperature migration tests may cause the mass transfer of carbon nanoparticles due to physical or other changes in the

film sample. Table 1 summarises the migration conditions and the food simulants applied in this study.

Swelling test

For the swelling experiment, dry PLA-based film samples were weighed and fully immersed in food simulant. The film was stored in the simulant liquids at the three time-temperature migration conditions, as listed in Table 1. The weight of the swollen sample was measured after blotting excessive solvent gently with filter paper. Sample weights before and after exposure to food simulants were used to conclude if any diffusion took place. The films were weighed in a high-precision analytical balance to ± 0.00001 g. The overall mass transfer due to swelling/dissolution was presented in $\mu\text{g cm}^{-2}$ of the film surface immersed in the simulant. The degree of swelling was calculated by the relative change ($S\%$) of mass of the film. The decrease of mass after swelling test was associated with the dissolution ($-S\%$). All these tests were performed in triplicate and the overall swelling/migration was calculated as the average.

Laser diffraction analysis

The detection of the migrants in the food simulants was performed by a laser diffraction analyser Analysette 22 Nano Tec plus (FRITSCH, Idar-Oberstein, Germany), using a wet dispersion unit, with an LOD of 0.020–2000 μm . The device allows the determination of particle-size distributions together with recognition of the particle shape in a single process. A new type of sensor allows recording of the areas of the diffraction pattern in which the information about the shape of the particles is contained. The result of the measurement is the average elongation calculated from the axis relation of an ellipsoid approximating the particles. The software allows the determination of the elongation ratios for the $\times 50$ value of a previously measured distribution

Table 1. Migration testing conditions and food simulants applied in this study.

Test	Migration testing conditions in this study	Food simulants
1	10 days at 40°C (standard static test)	10% v/v ethanol (simulant A) 3% v/v acetic acid (simulant B) 50% v/v ethanol (simulant D1) Olive oil (simulant D2)
2	4 h at 90°C (high-temperature static test)	
3	4 h at 90°C, and subsequent storage for 10 days at 40°C, including dynamic treatment 5 min daily (combined high-temperature-long-term dynamic test)	

for the calibration of shape, this providing transformation of the original equivalent volume-based data to number percentage (n %). After the migration test, 30 ml simulant solution were added into the wet dispersion unit and gently stirred during the laser analysis. In order to obtain repeatable and reliable results, all measurements were repeated at least three times with a different combination of optical parameters. Sub-micrometre and micrometre size migrants within the test range from 100 nm to 100 μm were analysed; the results are presented as a histogram representing the number of detected particles in number percentage (n %) versus particle size (μm). The laser diffraction analysis was applied for ethanol and acetic acid food simulants A, B and D1, but the device cannot be used for characterisation of the oil-based food simulant D2.

Electron microscopy

A TEM at an accelerating voltage of 200 kV was used for the analysis of the colloids of migrated nanoparticles into the food simulants. For this study a preliminary preparation technique was applied. A micro-quantity of colloid was dropped on standard copper TEM grid covered by a membrane from amorphous carbon; after that the grid was dried in a dust-free atmosphere at ambient conditions and then visualised at different magnifications. The phase composition of the dried colloids was determined by the selected area electron diffraction (SAED) mode of the microscope. Because of the specificity of the test, only simulants A, B and D1 were dried and subjected to TEM analysis.

An SEM Philips 515 at accelerating voltages of 25 and 5 kV was used to study the film surface morphology. Before the examination in the microscope, the samples were covered with metal coating for better conductivity of the surface and to avoid the discharge effects. The neat PLA and the PLA/GR/NC films before and after migration tests in the four food simulants (A, B, D1 and D2) were subjected to the SEM surface analysis.

Differential scanning calorimetry (DSC)

Calorimetric analysis was performed by a DSC Q20 (TA Instruments) in a nitrogen atmosphere, with double cycle of heating from 30 to 200°C at 10°

C min^{-1} separated by a single cooling cycle at 10° C min^{-1} . A sample of about 5 mg was put in an aluminium pan for the DSC analysis. The glass transition temperature (T_g), the crystallisation temperature (T_c), the total crystallinity (χ %) and the melting temperature (T_m) were determined from the first- and second-run DSC curves and their first derivative. The neat PLA and the PLA/GR/NC films before and after migration tests in the four food simulants were subjected to the DSC test.

Results and discussion

Swelling of PLA and PLA/GR/NC films in food simulants

The sorption and desorption processes in the film during migration tests were characterised by control on the swelling of the films in the three food simulants: 10% ethanol (A), 3% acetic acid (B) and 50% ethanol (D1), as varying time–temperature conditions. Table 2 summarises both the means of equilibrium percentage swelling (S %), or dissolution ($-S$ %) and the total concentration of migrant ($\mu\text{g cm}^{-2}$) from a 14- cm^2 film surface after the standard static and high-temperature static migration regimes. The repeatability of the measurements is given by the standard deviation of the results of the movement in and out of the film of an unspecified mixture of substances and simulant.

As seen from Table 2, the swelling of the neat PLA and the nanocomposite PLA/GR/NC films by the food simulants was not measurable (by weighing with a precision of ± 0.01 mg) after 10 days of storage at 40°C (standard static test) in the ethanol-based solutions (food simulants A and D1). The 3% acetic acid (simulant B) to a minor extent diffuses into the composite films in terms of sorption ($S = 0.84\%$), i.e., movement of the simulating liquid in the film. By contrast, a decrease of mass of the films was observed after 4 h storage at 90°C (high-temperature static test) in the three food simulants A, B and D1, which was associated with polymer dissolution ($S = -1.36\%$ to -2.37%). The release of mass from the neat PLA film is measured from 18 to 42 $\mu\text{g cm}^{-2}$, depending on the food simulant, while, 24–49 $\mu\text{g cm}^{-2}$ are released from the PLA/GR/NC film. We consider the release of nanoparticles from the films in the high-temperature migration test to be primary determined by the diffusion of

Table 2. Means of percentage equilibrium swelling and mass transfer from the neat PLA and the nanocomposite PLA/GR/NC films into food simulants A, B and D1. Migrants from 14 cm² contact film surface are estimated.

Test	Migration testing conditions	Food simulants	Neat PLA film			Nanocomposite PLA/GR/NC film			
			Swelling, dissolution, <i>S</i> %, (<i>-S</i> %)	PLA migrants ($\mu\text{g cm}^{-2}$)	Standard deviation migrants	Swelling, dissolution, <i>S</i> %, (<i>-S</i> %)	Total migrants ($\mu\text{g cm}^{-2}$)	Standard deviation migrants	Nanoparticle migrants ($\mu\text{g cm}^{-2}$) ^a
1	After 10 days at 40°C; standard static test	10% ethanol; simulant A	0	0	0	0	–	0	–
		3% acetic acid; simulant B	0	0	0	0.84	–	±0.010	–
		50% ethanol; simulant D1	0	0	0	0	–	0	–
2	After 4 h at 90°C; high-temperature static test	10% ethanol; simulant A	–1.65	21	±0.24	–1.95	28	±0.38	7.0
		3% acetic acid; simulant B	–2.37	42	±0.10	–2.99	49	±0.25	7.0
		50% ethanol; simulant D1	–1.36	18	±0.12	–1.80	24	±0.39	6.0

Note: ^aIn the very first approximation the amount of nanoparticle migrants is calculated from the total migrants of the PLA/GR/NC composite film reduced by the migrants of the neat PLA film.

abyotropic GNPs due to the concentration gradient and enhanced molecular mobility of the PLA matrix, as well as to be supported by partial dissolution of some organic substances from the PLA polymer by the aggressive food simulants and their subsequent diffusion out of the film. As a very first approximation, the nanocarbon migrants were calculated as the higher amount of migrants from the nanocomposite PLA/GR/NC film, reduced by the amount of migrants from the neat PLA film. Thus, we assume that about 6–7 $\mu\text{g cm}^{-2}$ carbon nanoparticles may diffuse from the composite film into the food simulants (Table 2).

Our results for the dissolution of the neat PLA are similar to those found by Mutsuga et al. (2008), who reported that 49.63 $\mu\text{g cm}^{-2}$ of lactic acid products migrated from polylactide food-contact materials into 4% acetic acid and 20% ethanol when at 95°C for 2 h. The results were also confirmed by Jamshidian et al. (2010) who reported that different thermodynamic properties such as polarity and solubility of the solvents play an important role in the swelling and dissolution processes. In our case, the 3% acidic acid (simulant B) and 50% ethanol (simulant D1) were found to be more aggressive for the PLA polymer compared with 10% ethanol (simulants A).

The swelling/dissolution results give the total amount of the dissolved substances from the PLA films, but no detailed information about the movement of specific film ingredients such as organic substances or nanoparticles out of the polymer

film. Therefore, we characterised the migrants by other techniques, such as laser diffraction analysis and TEM.

Characterisation of migrants in the food simulant solutions

Migrants detected in food simulants after high-temperature static migration

Our study demonstrates that nanoparticle migrants from the nanocomposite PLA/GR/NC film were not detectable in three food simulants (A, B and D1) after the standard static migration test of 10 days at 40°C, at an LOD of 0.020 μm by laser diffraction analysis. However, during the high-temperature static migration test conditions (at 90°C for 4 h), carbon nanoparticle migrants indeed were detected by TEM and laser diffraction analysis to migrate at a different degree in the acidic and alcohol-based food simulants. The size distribution histograms and the TEM micrographs of the migrants are compared in Figures 2(a–f).

Figures 2(a–c) show laser-diffraction size-distribution histograms representing the number percentage (*n* %) of migrants versus their size. The size distribution was determined based on the fact that the spatial distribution of scattered light is a function of the particle size and shape of the analysed sample (Stojanovic & Markovic 2012). The diagrams compare migrants from both the nanocomposite PLA/GR/NC film (light bars) and the control neat PLA film (dark bars) within the size range 0.1–100 μm ,

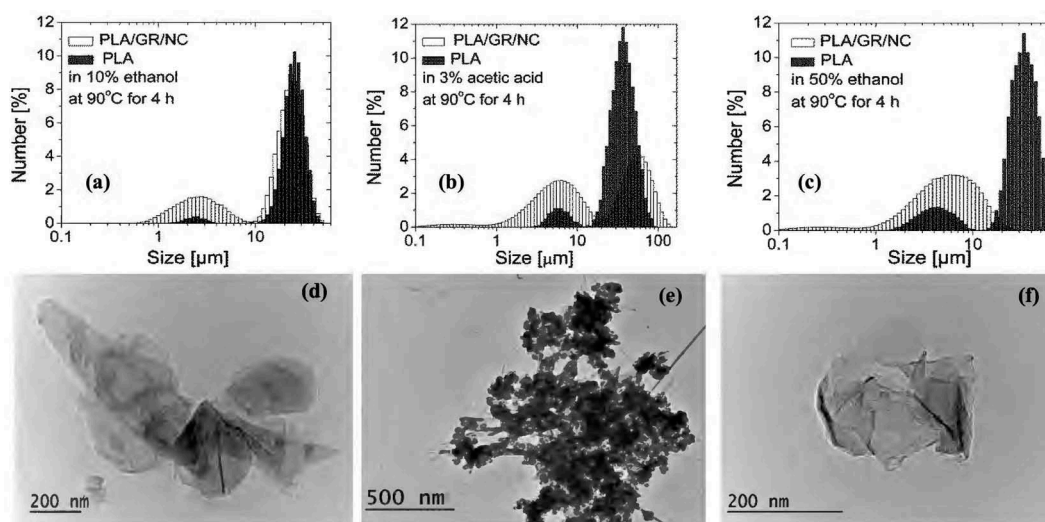


Figure 2. Size distribution histograms and TEM micrographs of migrants after high-temperature static migration test at 90°C for 4 h into three simulant solvents: (a, d) 10% ethanol; (b, e) 3% acetic acid; and (c, f) 50% ethanol. Histograms of the neat PLA film (dark bars) and nanocomposite PLA/GR/NC film (light bars) are compared. Corresponding TEM micrographs of dried simulants present the migrants from the PLA/GR/NC film.

detected in the three food simulants A, B and D1. The histograms for the neat PLA film have a bimodal size distribution of migrants with a small peak in the size range 1–10 μm and a sharp main peak within 10–100 μm ; this is associated with lactic-based substances hydrolysed from the PLA polymer in aqueous solvents (Mutsuga et al. 2008; Jamshidian et al. 2010). By contrast, migrants in nanoform appear for the composite PLA/GR/NC film in the size range 0.1–1 μm , which are of about 0.4 $n\%$ in simulants B and D, as well as about 0.2 $n\%$ in simulant A. Moreover, the micrometre size migrants from the PLA/GR/NC film within the range 1–20 μm are of an amount twice as high as those detected from the neat PLA. These might be associated with the release of carbon nanoparticles from the nanocomposite film, as such a result was not found from the neat PLA film.

When correlating the laser-diffraction analysis results in Figures 2(a–c) with the TEM micrographs in Figures 2(d–f), we consider that the detected migrants in nanoform in the three food simulants are primary nanoparticles of a few layers of graphene assembled in small aggregates of size above 200 nm–1 μm . This may be associated with the self-assembly of GNPs during drying of migrants before TEM analysis. Therefore, single GNPs of lateral size 100–200 nm are rarely detected in the TEM micrographs (Figures 2(d, f)).

Obviously, asymmetrical GNPs do indeed migrate from the PLA film into ethanol and acetic acid food simulants. Exceptionally, in the most aggressive 3% acetic acid (simulant B) not only graphene but also carbon nanotubes fixed in agglomerates with organic substances (Figure 2(e)) appear as migrants from the PLA/GR/NC film. The concentration of carbon nanofiller in the polymer is also of importance. The fullerenes and CB impurities are at very low concentration in the polymer, therefore they are detected rarely in the most aggressive acetic acid food simulant.

Figures 3(a–d) characterise the variety of nanoscale structures identified as migrants in the 3% acetic acid (simulant B) after the high-temperature static migration test. The intercept in the micrographs presents the SEAD patterns identifying different crystalline allotropes of carbon. In Figure 3(a), the TEM micrograph visualises a few layers of graphene platelets, and the electron-diffraction pattern confirms the graphite 2H, hexagonal phase with interplanar distance $d_{100} = 2.1390$ and $d_{110} = 1.2350$ Å (according to PDF 75-1621; ICDD, 2001), as well as lattice-spacing values $a = b = 2.47$ and $c = 6.79$, identifying graphene in agreement with Bosak and Krisch (2007).

In Figure 3(b), short carbon nanotubes of about 20 nm outer diameter, about 3 nm inner diameter and length above 100 nm are visible to release in

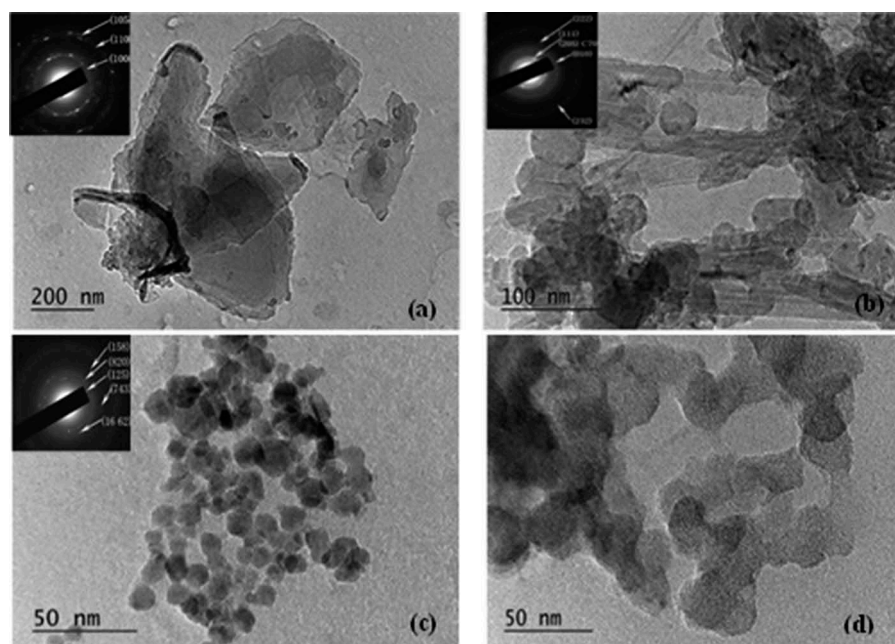


Figure 3. TEM micrographs of different migrants from nanocomposite PLA/GR/NC film identified in the food simulant B (3% acetic acid) after the high-temperature static test at 90°C for 4 h: (a) graphene; (b) carbon nanotubes; (c) fullerene-like C60/C70 crystals; and (d) carbon black. The intercepts present the electron diffraction patterns of different crystalline allotropes of carbon.

agglomerates fixed with organic substances. The SEAD pattern identifies these crystalline carbon allotropes as MWCNTs (Lucas et al. 1998). In Figure 3 (c), fairly monodisperse clusters of 5–10 nm compacted in a loose agglomerate of size about 100–200 nm are detected and the SEAD pattern shows that clusters are polycrystalline in nature and phase determined is C60 and C70 (Lucas et al. 1998; Deguchi et al. 2001). Finally, Figure 3(d) visualises the amorphous CB migrants of average size around 30–50 nm.

Migrants detected after high-temperature–long-term dynamic migration

Migrants in the three simulating solutions after the high-temperature–long-term dynamic migration test (for 4 h at 90°C and subsequent storage for 10 days at 40°C, including 5 min/daily ultrasonic treatment) are analysed by laser diffraction analysis and TEM. Example results are shown in Figures 4(a–f), comparing laser diffraction histograms and TEM micrographs of migrants detected in the three food simulants.

The size distribution of migrants from both the nanocomposite PLA/GR/NC film (light bars) and the neat PLA film (dark bars) is compared for the

three food simulants A, B and D1 in Figures 4(a–c). Similar to the results from the high-temperature static migration test, here the migrants from the neat PLA film show a bimodal size distribution with two peaks within the size range 1–100 μm . By contrast, the nanoscale migrants obtained from the PLA/GR/NC film of size 0.1–1 μm appear in larger amounts (0.5–0.7 $n\%$) and the migrants of micro-metre size 1–20 μm in Figure 4(a–c) show more complex multimodal size distribution in a high-temperature–long-term dynamic migration test compared with those of the static test in Figures 2(a–c). The main peak of size distribution of migrants for the nanocomposite PLA/GR/NC film is shifted towards higher size compared with those of the neat PLA.

The corresponding TEM micrographs in Figures 4(d–f) show large amounts of single graphene platelets of wide size range (from nano- to micrometre scale) released into the three food simulants A, B and D1, obviously due to the facilitating effect of dynamic treatment on dissolution processes. Graphene migrants in 3% acetic acid and 50% ethanol release in a larger amount compared with 10% ethanol. The high-temperature–long-term dynamic test in the acetic acid simulant (B) also extracts not

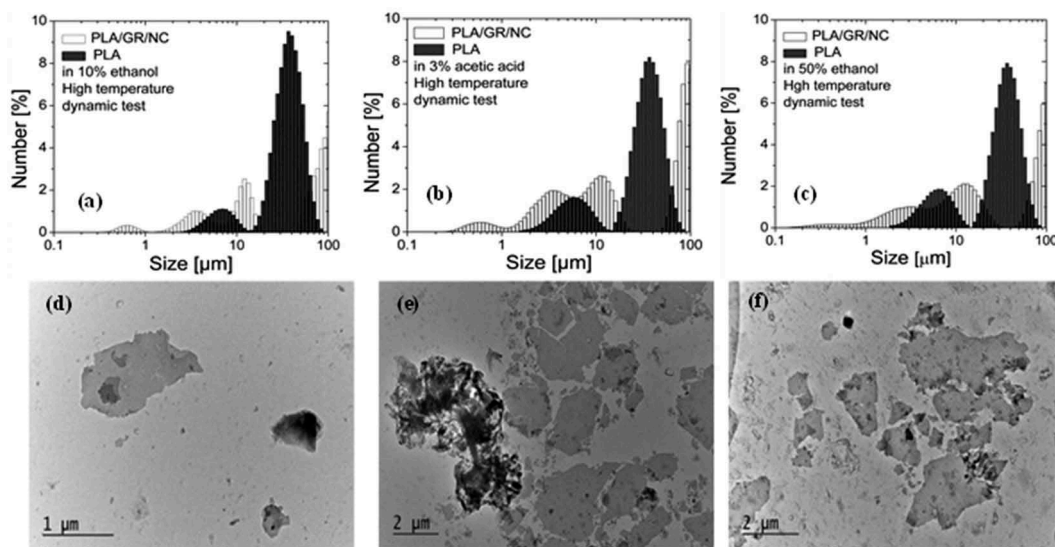


Figure 4. Size distribution histograms and corresponding TEM micrographs after high-temperature–long-term dynamic test. First row: comparison of the particle size distribution histograms of migrants from the neat PLA film (open bars) and the nanocomposite PLA/GR/NC film (full bars); second row: TEM micrographs of dried simulants of the PLA/GR/NC film in the three food simulants: (a, d) 10% ethanol (A), (b, e) 3% acetic acid (B), and (c, f) 50% ethanol (D1).

only graphene but also carbon nanotubes and other carbon nanoparticles that are mostly fixed with organic substances in large agglomerates around 10 μm (Figure 4(e)).

Characterisation of nanocomposite film integrity after migration tests

Thermal analysis

Calorimetric analysis was performed in order to characterise the structural changes of the polymer films produced by the migration tests. Figures 5(a, b) show example DSC thermograms (heat flow versus temperature), first run (a) and second run (b), of the

nanocomposite PLA/GR/NC film after the high-temperature–long-term dynamic migration test in the four food simulants: 10% ethanol (simulant A), 3% acetic acid (simulant B), 50% ethanol (simulant D1) and olive oil (simulant D2), compared with the control PLA/GR/NC film (before migration test). Table 3 summarises the thermal characteristics, such as: glass transition temperature (T_g), melting temperature (T_m), crystallisation temperature (T_c) and total crystallinity (χ %), determined from the first- and second-run thermograms.

In Figure 5(a), the DSC first-run thermogram of the control nanocomposite PLA/GR/NC film shows well-defined peaks for the glass transition

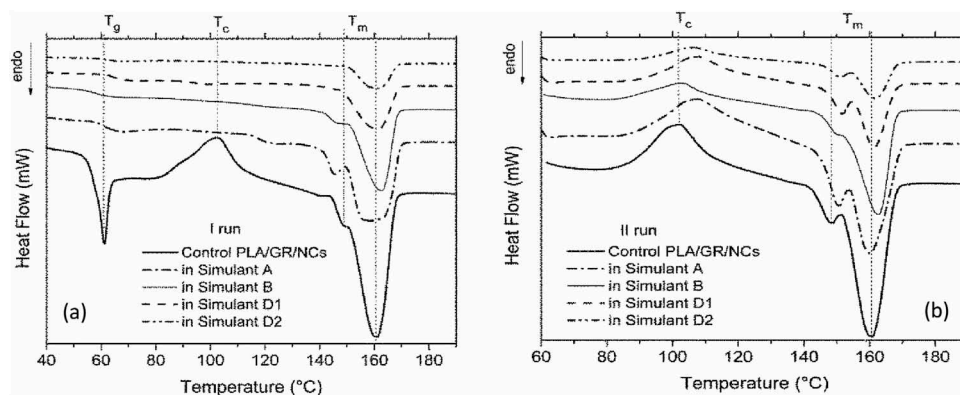


Figure 5. DSC thermograms from: (a) first run and (b) second run of the nanocomposite PLA/GR/NC film comparing control sample (first full line) and migrated films into four food simulants: simulant A (10% ethanol), simulant B (3% acetic acid), simulant D1 (50% ethanol) and simulant D2 (olive oil), after the high-temperature–long-term dynamic migration test.

Table 3. Thermal characteristics of PLA/GR/NC film from the first and second runs: glass transition (T_g), melting (T_m) and crystallisation (T_c) temperatures; total crystallinity (χ %) after high-temperature static migration tests in the food simulants: 10% ethanol (A), 3% acetic acid (B), 50% ethanol (D1) and olive oil (D2) compared with the control film.

Characteristics	Control film	Simulant B, 3% acetic acid	Simulant A, 10% ethanol	Simulant D1, 50% ethanol	Simulant D2, olive oil
T_g I run (°C)	61	–	–	–	–
T_c I run (°C)	103	–	–	–	–
T_c II run (°C)	102	102	107	108	107
χ I run (%)	9.99	–	–	–	–
χ II run (%)	7.49	4.28	5.73	4.55	4.73
T_m I run (°C)	148	146	145	–	–
	161	163	163	162	162
T_m II run (°C)	148	150	150	151	151
	161	163	161	162	162

temperature ($T_g = 61^\circ\text{C}$) and the crystallisation temperature ($T_c = 103^\circ\text{C}$) before the migration test. A double melting peak (T_m) was observed for the control film, where the large peak represents the melting temperature of the neat PLA polymer while the small peak might be associated with the melting of the PLA molecules attracted to the surfaces of carbon nanofillers. By contrast, the thermal characteristics of the migrated PLA/GR/NC films are changed after the high-temperature migration test in the four food simulating solutions. The T_g and T_c peaks disappear; the small T_m is shifted slightly towards lower temperatures (in simulants A and B) or has disappeared (in simulants D1 and D2) probably due to the swelling of the film by the simulant solutions.

The second-run DSC thermograms in Figure 5(b) show the presence of T_c for all films, but for the migrated films the crystallisation peak is shifted (with 5–6°C) towards higher temperatures compared with the control film. The small T_m is slightly shifted with 2–3°C to higher temperatures. The PLA total crystallinity (χ %) of the migrated films decreases about 1.3–1.75-fold in comparison with the control film due to the partial polymer dissolution. The effect of 3% acetic acid on the degree of crystallinity is slightly higher compared with other food simulants, probably due to the different degree of dissolution of the PLA polymer by the simulant solutions during the migration test.

In summary, the DSC analysis demonstrates a slight degradation of the crystal structure of the migrated films compared with the control film before migration test, which is associated with the partial dissolution of the PLA polymer into the food simulant solvents.

SEM analysis of the migrated film surfaces

SEM analysis was performed in order to examine the film surface before and after the high-temperature–

long-term dynamic migration test (4 h at 90°C , subsequent storage for 10 days at 40°C , including dynamic treatment) in the four food simulants. Figures 6(a–f) show example SEM micrographs for the migrated PLA/GR/NC film compared with the control film before migration. The control PLA/GR/NC film in Figure 6(a) has a smooth polymer surface without carbon nanoparticles on it, while in Figures 6(b) GNPs are visible on the surfaces of migrated films in 10% ethanol. Spheroidal bubbles and some small holes indicating diffusion of the dissolved PLA organic substances from the bulk towards the film surfaces are visible in Figure 6(c) for the migrated film in 3% acetic acid. By contrast, large amounts of graphene plates released on the film surfaces are observed after migration in 50% ethanol and olive oil (Figures 6(d–f)). Particularly, migrated PLA composite film in 50% ethanol shows a local degradation of the surface, pointed to by the white arrow in Figure 6(d). A higher magnification of this local area (Figure 6(e)) shows that the fibrous MWCNTs formed an entangled network as the PLA polymer matrix dissolves, which prevent their release into the food simulant.

Based on both DSC and SEM results, we consider that the partial dissolution of the PLA polymer by the aggressive simulant solutions at these severe migration conditions (of 90°C for 4 h followed by storage for 10 days at 40°C , including dynamic treatment) destroys locally the integrity of the film surface and large amounts of GNPs diffuse from the film volume on the surface. This effect strongly depends on the type of food simulant. Obviously, the enhanced dynamics of molecules above the glass transition facilitate the diffusion processes. Following the release mechanisms of nanoparticles proposed by Duncan and Pillai

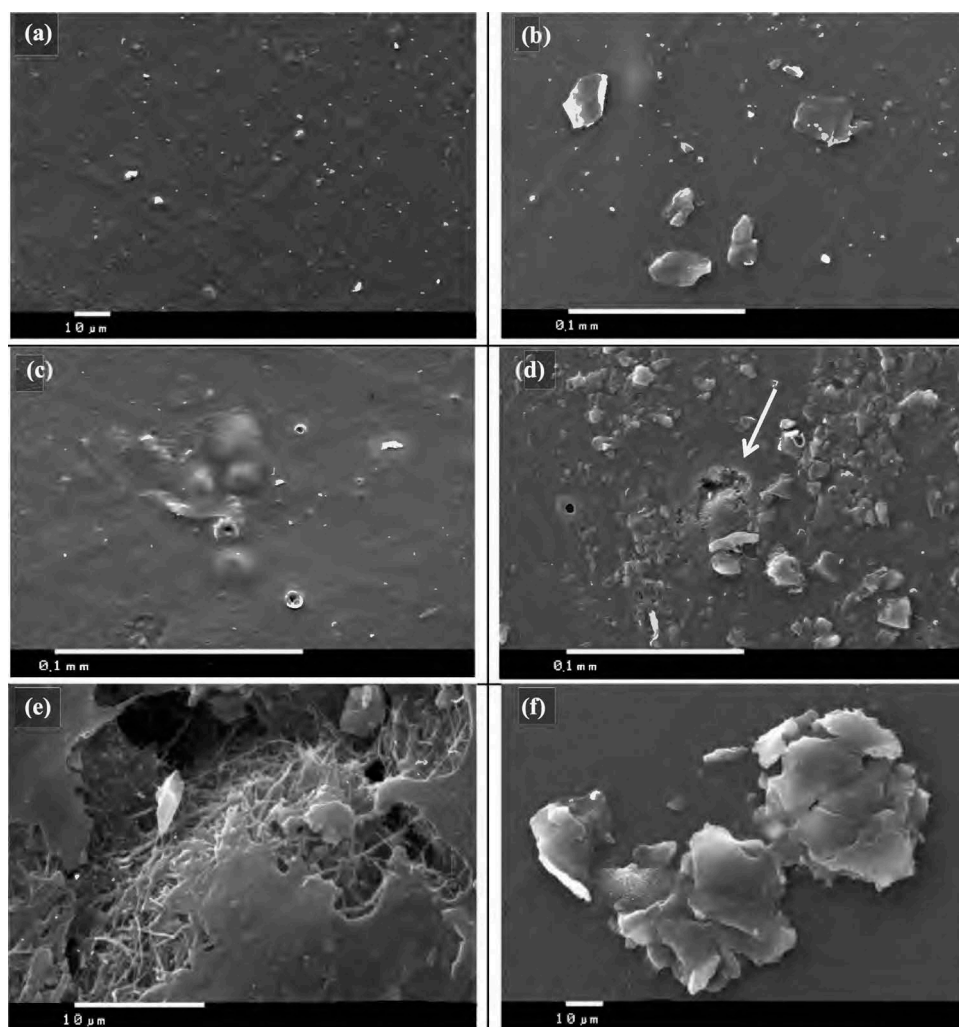


Figure 6. SEM micrographs of nanocomposite PLA/GR/NC film surfaces after high-temperature-long-term dynamic migration test: (a) control film; (b) in 10% ethanol; (c) in 3% acetic acid; (d, e) in 50% ethanol at low and high magnifications; and (f) in olive oil. Scale bars (white line) and size value are shown at the bottom of the images. The arrow in (d) points to a local degradation of the film surface, which is visualised in (e) at high magnifications.

(2015), we assume that such physical changes of the PLA polymer related to polymer dissolution provoke a diffusion of the dissolved organic substances doped with carbon nanoparticles, mainly graphene, out of the film towards the food simulant.

Safety concerns on graphene

GNPs detected as the main nanoscale migrant from the PLA/GR/NC film fall under the European Union-adopted definition 2011/696/EU (EU 2011a) of nanomaterial having one or more external dimensions in the size range 1–100 nm. According to (EC) No. 450/2009 and (EC) No. 10/2011, substances in nanoform should

only be used in active and intelligent plastic food-contact materials when they are explicitly authorised and included in the European Plastics Regulation specifications (Ebnesajjad 2013). Currently, only CB, TiN and SiO₂ are approved (with some restrictions) as nanomaterials for safety use in contact with food.

If one considers the PLA-matrix polymer, several authors observed that the migrated organic ingredients from the neat PLA films do not exceed the OML of substances from food packaging materials (OML = 10 mg dm⁻²) established by current European Union legal standards (Mutsuga et al. 2008; Mattioli et al. 2013). According to Conn et al. (1995), the migration of the PLA degradation products is not causing safety concerns as these

products will be subsequently hydrolysed in aqueous systems to lactic acid, which is a natural product and food ingredient. However, safety concerns may arise from GNPs that may release from the nanocomposite PLA-based films when used as food-contact materials.

In our study it was determined that the total amount of migrants from the investigated 2 wt% nanocomposite PLA/GR/NC film into the food simulants A, B and D1 at the high-temperature migration test (90°C for 4 h) are of 28, 49 and 24 $\mu\text{g cm}^{-2}$ respectively. In a first approximation the calculated amount of the migrated carbon nanoparticles is small, about 6–7 $\mu\text{g cm}^{-2}$ (Table 2). Our results show that the migrants from the investigated PLA composite films are small amounts of naked GNPs (of lateral size 100–1000 nm) and rarely MWCNTs fixed in plastic fragments that would probably not give rise to safety concerns in short-term exposure. As the toxicity of the naked GNPs would be different from the MWCNT/plastic fragments, the risk from the long-term exposure of consumers to GNPs via high-temperature processed packaged food cannot be ignored.

GNPs, as a novel composite additive, have recently been subjected to intensive studies for negative human and environmental impact (Smolander & Chaudhry 2010). The risk of graphene during its life cycle is rarely discussed in the literature (Arvidsson et al. 2013). The few peer-reviewed publications related to the toxic effects from exposure to graphene show that the shape, high surface area, surface chemistry and purity may lead to unknown toxicological effects and uncertainties on consumer safety (Ahmed & Rodrigues 2013; Arvidsson et al. 2013; Singh 2016). Researchers agree that graphene toxicity might be lower compared with the toxicity of carbon nanotubes (Ruiz et al. 2011, Guo & Mei 2014). Concerning the risk from the migration of graphene from packaged film into food or drink, little information is currently available concerning the uptake of nanoparticles following oral exposure by ingestion directly from food and drink (Jani et al. 1990).

Life cycle analysis (LCA) provides information in relation to exposure, as well as analysis of nanoparticles' release and monitoring throughout the whole product life cycle (Sweet & Strohm 2006). Life cycle behaviour of graphene remains at the very early

stages of development. Arvidsson et al. (2014) demonstrate the possibility of conducting a life cycle assessment study based mainly on information from patents and scientific articles on graphene production for use in composite bulk materials. The results show that the ultrasonication production route has lower energy and water use, but higher human and ecotoxicity impacts, compared with the chemical reduction route. For the time being, the available LCA studies and environmental assessments support the further development of bio-based polymers, however researchers agree that the effect of nanofillers on the environment has to be considered on a case-by-case basis (Patel et al. 2005; Hottle et al. 2013). More studies are needed on the effects of graphene as an additive in PLA composites for food packaging application, as well as on the distribution of PLA/graphene packaging materials in the waste stream in order to analyse their life cycle environmental impacts and to draw a prognosis hazard of graphene for humans and environment.

Conclusions

The study presents important findings indicating that GNPs of about 100–1000 nm in length and a few nanometres in thickness are indeed released from the investigated PLA composite film doped with 2 wt% carbon nanofillers under rather extreme migration conditions of 90°C for 4 h in the food simulants 10% ethanol, 3% acetic acid and 50% ethanol.

We assume that the following main factors allow the release of GNPs from the investigated composite PLA/GR/NC film: (1) the molecular mobility of the PLA matrix is significantly enhanced at the migration conditions of 90°C for 4 h, that is, above the glass transition of the neat PLA ($T_g = 61^\circ\text{C}$); (2) the enhanced dynamics of molecules of the matrix polymer facilitate the diffusion of GNPs, which are flexible sheets of a few nanometres thickness, therefore they have high mobility; and (3) the matrix PLA polymer is slightly dissolved during the migration conditions (the mass loss for the neat PLA vary within 1.36–2.37% depending on the food simulant), which also support the diffusion of the GNPs.

The fibrous MWCNTs formed entangled networks on the film surfaces as the PLA polymer

matrix dissolves, which prevents their release as single nanoparticles into the food simulants. The impurities of fullerenes (5–10 nm) and CB nanoparticles (about 30 nm) were of insignificant concentration in the nanocomposite film, therefore their migration was low or undetectable.

The possible release of GNPs from the PLA-based nanocomposite films into foodstuff has to be taken into account during the high-temperature processing of packaged food and subsequent long-time storage and transportation in order to predict the risk from graphene in the food chain over long-term exposure.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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